Clinical Validation of an Alternative Specimen Collection Kit for SARS-CoV-2 Testing at Fox Chase Cancer Center

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ABSTRACT

Background: Supply chain disruptions during the COVID-19 pandemic have affected the availability of components for specimen collection kits to detect SARS-CoV-2. Plastic injection molding offers a rapid and cheap method for mass production of swabs for upper respiratory tract sampling. Local production of virus transport medium increases flexibility to assemble sample collection kits if the medium provides appropriate stability for SARS-CoV-2 detection. Methods: A locally produced virus transport medium and a novel injection molded plastic swab were validated for SARS-CoV-2 detection by reverse-transcription quantitative polymerase chain reaction. Both components were compared to standard counterparts using viral reference material and representative patient samples. Results: Clinical testing showed no significant differences between molded and flocked swabs. Commercial and in-house virus transport media provided stable test results for over 40 days of specimen storage and showed no differences in test results using patient samples. Conclusions: This collection kit provides new supply chain options for SARS-CoV-2 testing.

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INTRODUCTION

Medical supply chain disruptions during the COVID-19 pandemic have affected the availability of a wide range of products and services, including the components of specimen collection kits used to detect SARS-CoV-2 infection. While the early shortages of virus transport medium (VTM) and flocked collection swabs[1],

[2] have eased, it remains prudent to plan for the possibility of new disruptions and identify alternative kit components that provide testing laboratories flexibility for local or independent sourcing. This report describes institutional experience at the Fox Chase Cancer Center—Temple University Health System in validating locally produced M4RTalt VTM and an injection molded sampling swab for SARS-CoV-2 clinical diagnostic testing by reverse-transcription quantitative polymerase chain reaction (RT-qPCR).

Specimen collection swabs for sampling the upper respiratory tract are generally constructed with cotton flocking, synthetic flocking, or a synthetic sponge attached to a plastic shaft. A variety of shaft lengths are available for oropharyngeal (OP) or anterior nares (AN) collection, and longer, more flexible swab heads are used for nasopharyngeal (NP) sampling. Immediately after specimen collection, the swab head is placed in 1-3 mL of VTM, which releases the material out of the swab and into a liquid that stabilizes virus particles, preserving nucleic acids and supporting short-term storage at 4 °C or long-term freezing. Viral detection in saliva is another option that may be performed with or without a swab, and several meta-analyses are available comparing SARS-CoV-2 testing using OP, AN, NP, and saliva specimens.[3],[4],[5]

In response to supply shortages, microbiology and cell culture laboratories have worked with molecular diagnostics colleagues to locally produce VTM. For example, the Philadelphia Veteran Affairs Medical Center and Harvard Medical School have reported production and quality control processes for VTM containing Hanks Balanced Salt Solution (HBSS), phenol red, fetal bovine serum (FBS), gentamicin sulfate, and amphotericin B.[6],[7] The local production of swabs has been described, for example, using filament-based 3D printing to manufacture 50 swabs in less than 4 hours,[8] and a number of 3D printing designs and regulatory considerations have been reviewed.[9],[10],[11]

METHODS

M4RTalt is a simplified VTM based on Remel MicroTest M4RT Multi-Microbe Media (Thermo Fisher Scientific R12506). It contains 1x HBSS, 100 ug/mL gentamicin sulfate, 1 ug/mL amphotericin B, 1% bovine serum albumin (BSA), 10 mM HEPES pH 7.3, 0.2 M sucrose, and 2 mM L-glutamine. M4RTalt is sterilized by 0.22 um filtration and stored at 4 °C for up to 6 months. Aliquots of 1 or 3 mL were placed in sterile conical bottom screw cap tubes before distribution to clinical collection sites, where they were kept at 4 °C before and after specimen collection.

Solid plastic swabs were manufactured by injection molding on a precision injection molding work cell. Swab dimensions are 145 mm total length with a 3-mm shaft width and a tapered 5-mm collection head patterned with indentations to increase surface area and trap liquid. The shaft is scored to provide a breakpoint that reduces the swab length to 100 mm after insertion into the VTM tube. The prototype batch was received in bulk, and then swabs were individually wrapped in aluminum foil and sterilized by autoclaving. Commercial versions will have various shaft lengths and be available as individually wrapped, pre-sterilized units.

The saturated volume of liquid retained by molded swabs, HydraFlock flocked swabs (Puritan Medical Products 25-3706-H), and BD BB CultureSwab EZ sponge swabs (Becton, Dickinson and Co 220144, manufactured by Copan Italia SpA) was measured by placing 3 replicates of each swab in 1 mL VTM for 1 minute and then cutting the shaft and placing the swab head up in a microcentrifuge tube for centrifugation at 20,000 x g for 1 min. The volume of released VTM was measured using micropipettes. Molded and flocked swabs were also tested for the collection of the virus by placing 6 swabs of each type in 1 mL of VTM containing heat-inactivated SARS-CoV-2 (ATCC VR-1986HK) at 1.9 x 10^7 virions/mL and then transferring the swab to 1 mL sterile VTM for 4 hours (3 replicates) or 18 hours (3 replicates) at 4 ^{o}C.

Viral RNA was extracted from 300 uL VTM using chemagic Viral DNA/RNA 300 Kit H96 magnetic purification reagents (PerkinElmer CMG-1033-S) and a chemagic 360 instrument with 96-rod head (PerkinElmer 2024-0020). The manufacturer's protocol was followed without modification for RNA purification; each sample was spiked with 3.5 uL MS2 bacteriophage control before lysis and purification in a 96-well format using chemagic 360 script Viral300 VD200309 and elution in 60 uL chemagic elution buffer. Development and validation assays were conducted using the AccuPlex SARS-CoV-2 Reference Material Kit v2 (SeraCare 0505-0133), an encapsulated recombinant alphavirus carrying SARS-CoV-2 genomic segments, and provided at 5,000 genomic copies/mL.

RT-qPCR was performed using the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific A47814). A modified reaction master mix was prepared containing 7.5 uL 1-step Multiplex MasterMix, 1.5 uL COVID-19 Realtime PCR Assay Multiplex, and 3 uL nuclease-free water per reaction. The reaction master mix was aliquoted (12 uL per well) to MicroAmp Fast Optical 96-well plates (Thermo Fisher Scientific 43-669-32) and mixed with 18 uL of RNA sample. Each plate included a run-to-run control sample, SARS-CoV-2 positive control, and no-template negative control. Plates were sealed with MicroAmp Optical Adhesive Film (Thermo Fisher Scientific 43-119-71) and placed in a QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher). The PCR cycling profile was programmed as described in the TaqPath COVID-19 instructions (2 minutes at 25 ^{o}C, 10 minutes at 53 ^{o}C, and 2 minutes at 95 ^{o}C followed by 40 cycles of 3 seconds at 95 ^{o}C and 30 seconds at 60 ^{o}C). To analyze VTM stability, RNA was extracted from specimen aliquots on the indicated days and stored at -80 ^{o}C until the full set was assembled for RT-qPCR in 1 assay batch.

Real-time PCR data were collected as instructed in the TaqPath COVID-19 kit for the SARS-CoV-2 N, S, and ORF1ab genes and for the MS2 control; data were processed with the auto-threshold algorithm in QuantStudio 12K Flex Software (v1.4). A QuantStudio Amp Score of 2.2 or greater (a measure of the quality of the amplification curve for each analyte gene) was required for inclusion of data points. Test results of Positive, Negative, Indeterminate, or Failed were assigned as described in the kit instructions. Additionally, a quantitative signal value was computed by normalization to the MS2 spiked control by subtracting the average of N, S, and ORF1ab threshold cycles (Ct) from the Ct for MS2 and then adding an arbitrary 100 units. Higher

signal therefore reflects more virus present in the sample and is normalized for relative comparisons between samples and batches.

Data analysis tests for statistical significance included t-Test: Two-Sample Assuming Equal Variances in Microsoft Excel and McNemar's test.[12],[13]

M4RTalt, chemagic RNA extraction, and modified TaqPath RT-qPCR were incorporated into the Fox Chase Cancer Center Molecular Diagnostics Laboratory MDL-SARS-CoV-2 test, and the test's performance was validated using Food and Drug Administration (FDA)-recommended protocols for assessing the limit of detection and diagnostic concordance. These data were reviewed by the FDA in an application for emergency use authorization, and the test was determined to be a laboratory developed test (LDT) not subject to further FDA designation. The MDL-SARS-CoV-2 test is performed in a laboratory accredited for high-complexity molecular diagnostics in compliance with College of American Pathology regulations and proficiency testing for an LDT.

RESULTS

Clinical matrix was prepared by pooling leftover samples from patients who tested negative in the Fox Chase outpatient screening clinic. Two types of matrix were prepared: commercial VTM (BD Universal Viral Transport 220526) that had contained a flocked NP swab and M4RTalt that had contained a flocked OP swab. A dilution series of the SARS-CoV-2 reference material was made in both matrices and tested by RT-qPCR for a limit of detection (LOD) range finding. The analyte ranged from 2,500 to 0 genome copies/mL VTM, and the LOD was determined to be 100 copies/mL (30 copies per RNA extraction, 9 copies per PCR assuming 100% RNA recovery) (Supplementary Tables 1-2). This LOD was confirmed in 24 replicate samples for BD and M4RTalt VTM (Table 1).

Table 1			
Test outcomes for 24 replicate samples containing a SARS-CoV-2 reference at the limit of detection in a clinical matrix of BD or M4RTalt VTM			
LOD sample	Test result		
	Positive	Negative	Indeterminate
NP + commercial VTM	24	0	0
OP + M4RTalt	24	0	0

Leftover screening samples from patients who were positive for SARS-CoV-2 were stored at 4 ^{o}C and repeatedly tested to measure analyte stability over a time course. Flocked swab specimens were collected in 3

mL BD or M4RTalt VTM. Five BD and 5 M4RTalt samples showed uniform RT-qPCR signal values for over 40 days; a sixth M4RTalt sample, which originally had low signal, tested negative at 2 time points (Figure 1).
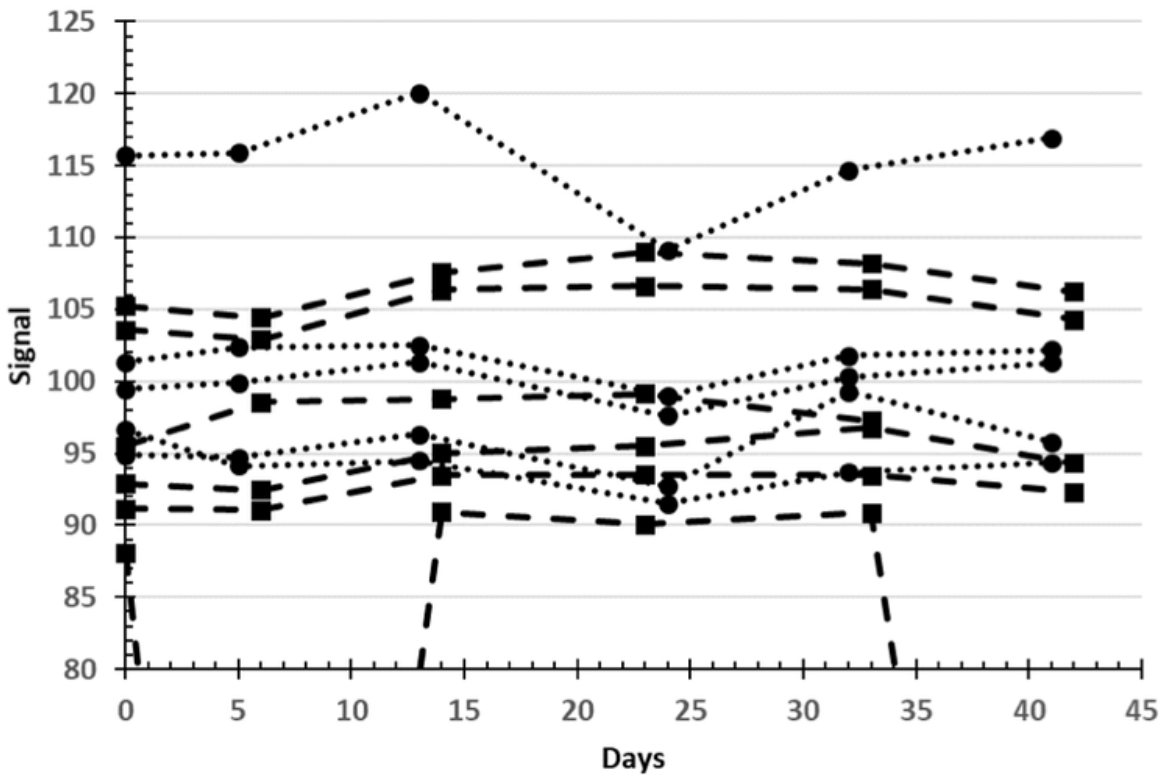

Figure 1

Viral stability in VTM over time. Patient samples positive for SARS-CoV-2 (circles and squares at day 0) were collected and stored in BD (dotted lines) or M4RTalt (dashed lines) VTM at 4 ^{O}C. The day 0 diagnostic samples were repeatedly tested by RT-qPCR on the days indicated to measure the SARS-CoV-2 signal.

Molded, flocked, and sponge swabs (Figure 2) were placed in 1 mL VTM or used to take AN specimens from a healthy control to determine the maximum saturation volume and typical clinical volume of liquid collected, respectively (Table 2). The ability of molded and flocked swabs to collect the virus was also compared using a SARS-CoV-2 reference material. After saturation in the reference sample, 3 replicates of each swab type were placed in sterile VTM for 4 or 18 hours to elute the collected virus (Table 3). While no significant differences were observed between elution times, the average signal was significantly different (t-test $P < 0.05$) between swab types, reflecting their differences in saturated volume collected.
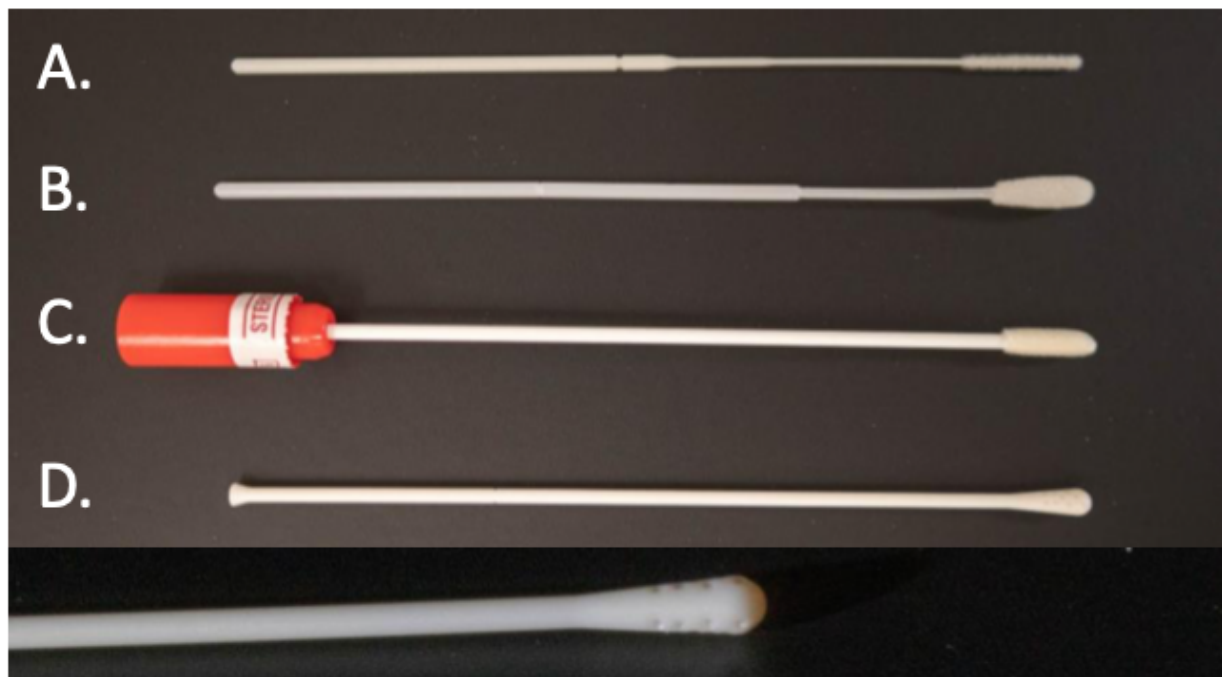
Figure 2
Four examples of collection swabs used in the study. A commercial flocked NP swab (A) and
flocked (B) or sponge (C) swabs for OP and AN collection are shown next to a prototype
injection molded swab (D) with dimpled head.

Table 2						
Volume of liquid recovered after VTM saturation or AN collection for 3 replicates of 3 types of swabs						
	Average volume: uL (SD)					
Source	Flocked		Sponge		Molded	
VTM	151.3	(19.6)	26.7	(7.6)	7.1	(1.5)
AN	54.3	(11.2)	22.0	(9.1)	2.6	(0.5)
SD, standard deviation.						

Table 3
SARS-CoV-2 test signal from 3 replicates of flocked or molded swabs saturated with a viral reference sample and eluted in VTM for 4 or 18 hours

	Average signal (SD)				
Elution hours	Flocked		Molded		P (t-test)
4	108.6	(0.27)	99.5	(0.37)	2.2×10^{-6}
18	108.8	(0.43)	100.3	(1.03)	9.8×10^{-5}
SD, standard deviation.					

Outpatients from the Fox Chase screening clinic were tested with a flocked OP swab and a molded OP swab, both collected in M4RTalt. Diagnostic concordance between swab types was high (Figure 3a), with no statistically significant difference by McNemar's test. The quantitative signal was also not significantly different between swab types (Figure 3b). The evaluation was therefore expanded to patients admitted to Jeanes Hospital—Temple Health with COVID-19 symptoms. Diagnostic NP samples collected with flocked swabs and commercial VTM were taken at admission and tested at Temple University Hospital by the Roche cobas SARS-CoV-2 assay. Within 24 hours, patients self-collected a second sample from AN using molded swabs and commercial VTM, which was tested at Fox Chase. Diagnostic concordance remained high in this phase of the evaluation (Figure 3c), and the combined data showed no significant difference between molded and flocked swabs (McNemar's $P = 0.68$, Figure 3d).

A.

OP Molded	OP Flocked Positive	OP Flocked Negative	
Positive	6	2	P 0.48
Negative	0	15	

B.

Subject	SARS-CoV-2 Signal OP Flocked	SARS-CoV-2 Signal OP Molded	SARS-CoV-2 Signal Difference
1	93	90	-3
5	94	102	8
8	86	96	10
16	103	104	1
18	95	92	-3
27	93	92	-1

C.

AN Molded	NP Flocked Positive	NP Flocked Negative	
Positive	34	0	P 0.13
Negative	4	8	

D.

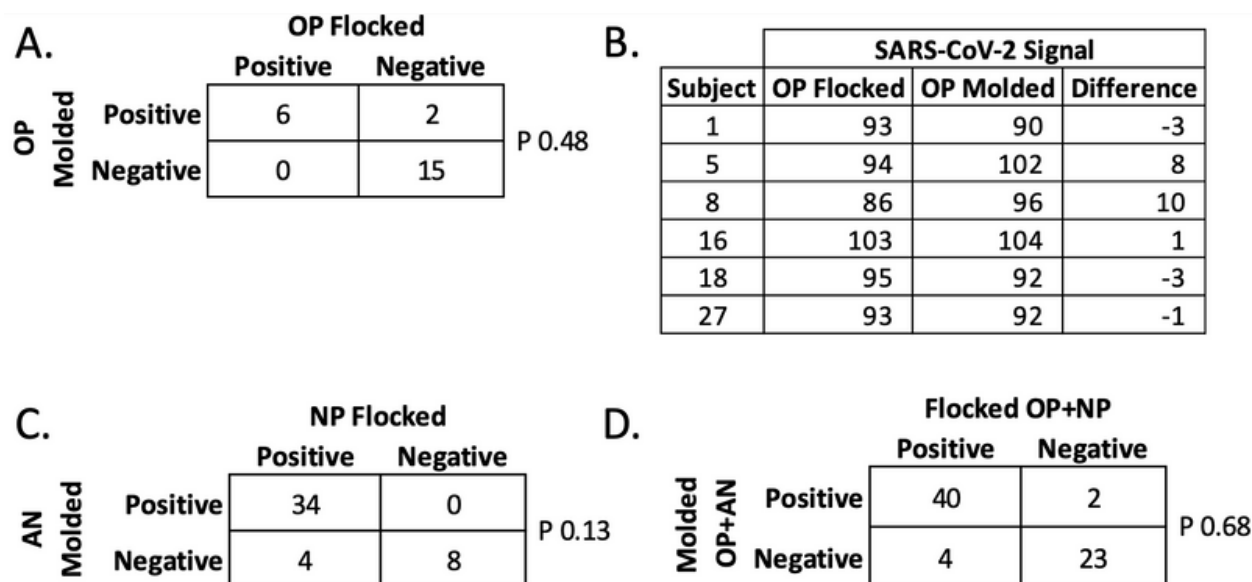| Molded OP+AN	Flocked OP+NP Positive	Flocked OP+NP Negative	
Positive	40	2	P 0.68
Negative	4	23	

Figure 3

Qualitative and quantitative test results from clinical samples. Two specimens per outpatient screening subject were consecutively collected by molded or flocked swabs with OP collection, and the results are shown for the diagnostic findings (A) and RT-qPCR signal (B). (C) An expanded cohort was tested at hospital admission using flocked swabs with NP collection and again within 24 hours using molded swabs and AN collection. (D) The combined diagnostic findings from sets A and C show no statistically significant difference between flocked and molded swabs. All P-values are from McNemar's test for case-control analyses.

DISCUSSION

During the development of our SARS-CoV-2 specimen collection kit, we considered M4RT medium to offer advantages over other locally produced VTMs including the use of HEPES buffer, BSA rather than FBS, and gelatin and sucrose as cryoprotectants. M4RTalt is a simplified version of M4RT, omitting the phenol red pH indicator and gelatin, and can be prepared from reagents commonly available in hospital and research laboratories familiar with microbiology or cell culture techniques. Since there was no intention for the VTM to be compatible with later culture conditions, the growth factors in FBS were not needed, and BSA, HBSS, and glutamic acid provide adequate virus stabilization. Short storage times between collection, RNA extraction, and refrigeration make bacterial overgrowth less of a concern, with added protection provided by 2 antibiotics.

High-concentration sucrose has supported long-term freezing at -80 $^{\circ}$C with no reduction in RT-qPCR SARS-CoV-2 signal (data not shown). M4RTalt performed no differently than commercial VTM in LOD experiments using viral reference material or in molecular diagnostic results with patient samples from clinical screening. RNA-based detection remained stable for over 40 days of storage at 4 $^{\circ}$C, a time frame much longer than typically needed for diagnostic testing to be completed.

Despite initial skepticism, we found that solid plastic swabs performed as well as flocked swabs in the clinical diagnostic setting. While the collected volumes of liquid are clearly lower, patients' viral loads are apparently more than enough to allow qualitative detection by RT-qPCR, perhaps even without quantitative penalty ([Figure 3](#)b). In our expanded patient cohort, we observed 4 cases that tested negative with molded swabs and positive with flocked swabs, but this discrepancy is confounded by anterior nares versus nasopharyngeal collection. Overall, there was no statistically significant diagnostic difference between molded and flocked swabs ([Figure 3](#)d).

During the COVID-19 pandemic, flocked and sponge swabs have remained in the domain of a limited number of specialty manufacturers who can manage the sourcing of multiple raw materials and their incorporation into relatively higher-complexity fabrication processes. 3D printing is a much newer manufacturing technology that can create swabs from a single feedstock but has limited worldwide availability and can be difficult to scale up without large capital expenditures. Injection molding is a mature technology that uses a single plastic feedstock abundantly available in bulk, is found in existing factories around the world, and can rapidly produce large quantities of swabs with short notice.

This evaluation employed a limited cohort of patients (<100) tested at 2 adjacent institutions during an early phase of the pandemic. A variety of VTMs are broadly used, so it is unlikely that expanded testing of M4RTalt would reveal significantly different results. However, molded swabs are a novel addition to this class of traditional medical devices, and additional comparisons at other locations is warranted. It will be important to test molded swab performance with other PCR-based assays and with non-PCR techniques that may have lower inherent sensitivities. A well-replicated comparison using molded swabs with AN, OP, and NP collection methods and other specimen types would be interesting as well as a survey of performance with recent SARS-CoV-2 variants.

ACKNOWLEDGMENTS

SUPPLEMENTAL MATERIAL

Supplemental Table 1

Ct and Amp Score values for LOD range finding using a series of SARS-CoV-2 recombinant alphavirus reference sample dilutions in M4RTalt clinical matrix.

CSV Table S1.csv   8 KB

Supplemental Table 2

Diagnostic test results for the LOD range finding using BD and M4RTalt clinical matrices.

CSV Table S2.csv   1 KB

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